Current Biology 19, 853–858, May 26, 2009 ©2009 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2009.03.063

Report

Decoding of Cytoplasmic Ca^{2+} Oscillations through the Spatial Signature Drives Gene Expression

Joseph Di Capite,[1] Siaw Wei Ng,[1] and Anant B. Parekh[1,*]
[1]Department of Physiology, Anatomy and Genetics
University of Oxford
Parks Road
Oxford OX1 3PT
UK

Summary

Cytoplasmic Ca^{2+} oscillations are a universal signaling mode that activates numerous cellular responses [1, 2]. Oscillations are considered the physiological mechanism of Ca^{2+} signaling because they occur at low levels of stimulus intensity [3]. Ca^{2+} oscillations are proposed to convey information in their amplitude and frequency, leading to activation of specific downstream targets [4–6]. Here, we report that the spatial Ca^{2+} gradient within the oscillation is key. Ca^{2+} oscillations in mast cells evoked over a range of agonist concentrations in the presence of external Ca^{2+} were indistinguishable from those in the absence of Ca^{2+} when plasmalemmal Ca^{2+} extrusion was suppressed. Nevertheless, only oscillations with accompanying Ca^{2+} entry through store-operated CRAC channels triggered gene expression. Increased cytoplasmic Ca^{2+} buffering prevented oscillations but not gene activation. Local Ca^{2+} influx and not global Ca^{2+} oscillations therefore drives gene expression at physiological levels of stimulation. Rather than serving to maintain Ca^{2+} oscillations by replenishing stores, we suggest that the role of oscillations might be to activate CRAC channels, thereby ensuring the generation of spatially restricted physiological Ca^{2+} signals driving gene activation. Furthermore, we show that the spatial profile of a Ca^{2+} oscillation provides a novel mechanism whereby a pleiotropic messenger specifically activates gene expression.

Results and Discussion

Since Prince and Berridge first proposed the existence of cytoplasmic Ca^{2+} oscillations in blowfly salivary gland [7], these oscillations induced by receptor activation have been found in virtually all cell types [8]. Ca^{2+} oscillations are thought to confer several advantages over a sustained bulk Ca^{2+} rise in activating Ca^{2+}-dependent responses [9]. These include increased sensitivity to the Ca^{2+} signal and circumvention both of the desensitization and excitotoxicity that can occur in response to a maintained Ca^{2+} signal. Furthermore, different amplitudes and/or frequencies of Ca^{2+} oscillation can recruit specific downstream Ca^{2+}-dependent targets [4–6, 10], providing a mechanism for selective responses to the promiscuous Ca^{2+} signal.

The diffusion of Ca^{2+} through an open Ca^{2+} channel in either the plasma membrane or an intracellular organelle results in the rapid build-up of a local microdomain of elevated Ca^{2+} [11]. Depending on the type of Ca^{2+} channel, the Ca^{2+} concentration within a microdomain can reach tens of micromolar, several-fold higher than the bulk cytoplasmic Ca^{2+} rise [11, 12]. Ca^{2+} microdomains associated with voltage-gated Ca^{2+} channels activate neurotransmitter release [11], open colocalized Ca^{2+}-dependent K^+ channels [13], and trigger gene transcription [14, 15], whereas microdomains that accompany the opening of store-operated Ca^{2+} release-activated Ca^{2+} (CRAC) channels activate the Ca^{2+}ATPase pump [16], adenylyl cyclase [17], endothelial nitric oxide synthase, and Ca^{2+}-dependent phospholipase A_2 [18]. Ca^{2+} microdomains arising from open $InsP_3$ receptors in the endoplasmic reticulum stimulate juxtaposed mitochondria to generate ATP [19]. Ca^{2+} oscillations arise from the opening of Ca^{2+}-permeable ion channels, so we hypothesized that the subcellular spatial profile of Ca^{2+} oscillations might play a role in activating selective downstream targets. If this is the case, then Ca^{2+} oscillations that are identical in amplitude and frequency should differentially activate downstream targets depending on the spatial location of the underlying Ca^{2+} channels. Here, we have generated Ca^{2+} oscillations that have identical amplitudes and frequencies but with distinct spatial profiles and we compared their abilities to activate gene expression.

To test the idea that the profile of a Ca^{2+} oscillation contained important information, we generated two kinds of $InsP_3$-driven Ca^{2+} oscillation with distinct spatial signatures: one involved Ca^{2+} release from the endoplasmic reticulum without any Ca^{2+} influx, whereas the other comprised Ca^{2+} release followed by Ca^{2+} influx through store-operated CRAC channels. Only the latter scenario would result in significant elevation of the subplasmalemmal Ca^{2+} concentration.

Mast cells express cell-surface cysteinyl leukotriene type I receptors that couple to phospholipase C and which are activated by the proinflammatory molecule leukotriene C_4 (LTC_4) [20]. Stimulation with a submaximal concentration of LTC_4 (160 nM) in the presence of external Ca^{2+} evoked a series of repetitive Ca^{2+} oscillations that were maintained for at least 800 s (Figure 1A, representative of $n > 500$ cells with ~98% of cells responding). In the absence of external Ca^{2+}, oscillations ran down with time such that most cells failed to generate a Ca^{2+} signal after 400 s (Figure 1B, $n > 350$ cells, with virtually all cells responding). Hence Ca^{2+} influx is needed to sustain the Ca^{2+} oscillations. The Ca^{2+} influx pathway involves the CRAC channel because (1) in whole-cell patch clamp experiments, LTC_4 activates CRAC channels via store depletion [20] and (2) block of these channels with 1 μM Gd^{3+} or La^{3+} results in rundown of the oscillations in the presence of external Ca^{2+} and at a rate similar to that seen in the absence of external Ca^{2+} (Figure S1 available online; 56 cells). Ca^{2+} oscillations run down in Ca^{2+}-free solution because the Ca^{2+} that is released from the endoplasmic reticulum by $InsP_3$ is transported out of the cell, resulting in gradual depletion of the Ca^{2+} store. Of the plasma membrane Ca^{2+} removal mechanisms, the Ca^{2+}ATPase pump is particularly effective at clearing Ca^{2+} from the cytoplasm in mast cells [21]. After block of this pump with La^{3+}, stimulation with LTC_4 in the absence of external Ca^{2+} resulted in Ca^{2+} oscillations that were now sustained (Figure 1C; labeled 0 Ca^{2+} + La^{3+}, $n > 300$ cells, with

*Correspondence: anant.parekh@dpag.ox.ac.uk

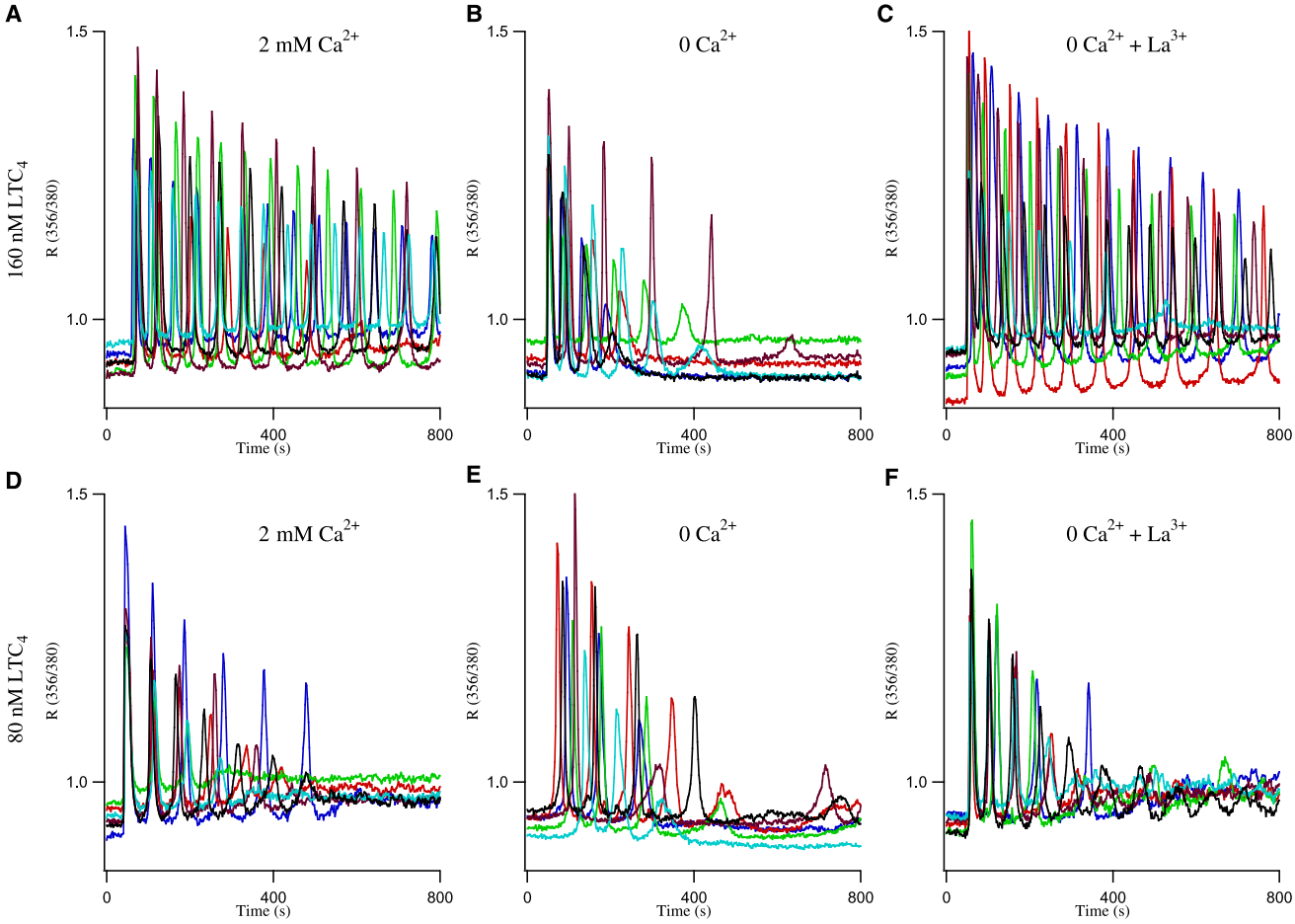

Figure 1. Cytoplasmic Oscillations Evoked by Low Levels of LTC_4

(A) Treatment with 160 nM LTC_4 evoked Ca^{2+} oscillations that were maintained in 2 mM external Ca^{2+}.

(B) Oscillations ran down in the absence of external Ca^{2+}.

(C) Oscillations were maintained in the absence of external Ca^{2+}, if the plasma membrane Ca^{2+}ATPase pump was blocked with La^{3+} (1 mM).

(D–F) Ca^{2+} oscillations evoked by a lower concentration of LTC_4 (60 nM) showed a similar pattern. Each graph depicts different cells in a field of view. Different colors correspond to different cells. In each panel, LTC_4 was added at 30 s.

~all cells responding). Because repetitive Ca^{2+} oscillations can be supported in the absence of Ca^{2+} influx, Ca^{2+} entry does not directly contribute to their generation. Rather, in agreement with a previous study [22], it is needed to refill the stores and thus maintain the oscillatory mechanism that is driven by the intracellular milieu.

At lower agonist concentrations, the pattern of Ca^{2+} oscillations was indistinguishable between cells stimulated in 2 mM external Ca^{2+}, 0 Ca^{2+}, and 0 Ca^{2+} together with La^{3+} (Figures 1D–1F compares these responses in cells exposed to a low dose of LTC_4), although responses in 0 Ca^{2+} tended to run down slightly more quickly. We analyzed the amplitude and frequency of the Ca^{2+} oscillations for three different LTC_4 concentrations (Figure 2, each point represents >200 cells). The amplitude and frequency of the Ca^{2+} oscillations to LTC_4 were generally similar when evoked in either 2 mM Ca^{2+} or 0 Ca^{2+} and La^{3+}, whereas responses in 0 Ca^{2+} were smaller and shorter lasting.

If distinct spatial Ca^{2+} gradients hidden within similar global Ca^{2+} oscillations encode important information, then one would predict these spatial profiles to differ in their ability to activate Ca^{2+}-dependent responses. To test this, we compared the ability of the Ca^{2+} oscillations to activate expression of the gene *c-fos* because (1) it is an important component of the transcription factor complex AP1 that regulates expression of chemokines in immune cells [23] and (2) Ca^{2+} influx through CRAC channels is a strong activator of *c-fos* transcription and translation [24]. Stimulation with LTC_4 for 12 min in the presence of external Ca^{2+} consistently activated c-*fos* expression over a range of agonist concentrations (Figure 3A). Strikingly, stimulation for the same time in the absence of Ca^{2+} failed to evoke any *c-fos* expression even when La^{3+} was present to maintain the Ca^{2+} oscillations (Figure 3A). This was the case over the range of LTC_4 concentrations tested (Figures 3B–3D). For identical amplitudes and frequencies, only Ca^{2+} oscillations in the presence of Ca^{2+} influx drove *c-fos* expression. Moreover, stimulation with 160 nM LTC_4 in Ca^{2+}-free solution evoked 7 ± 1 Ca^{2+} oscillations in the 12 min period, whereas 80 nM LTC_4 in 2 mM Ca^{2+} generated only 3.5 ± 0.7 oscillations. Despite this, gene expression occurred only after challenge with LTC_4 in the presence of external Ca^{2+}. The spatial Ca^{2+} gradient that arises from local Ca^{2+} influx through CRAC channels therefore has two important consequences: (1) it enables lower concentrations of an agonist to evoke a cellular response and (2) fewer Ca^{2+} oscillations are needed to activate gene expression, thus increasing the efficacy of excitation-transcription coupling.

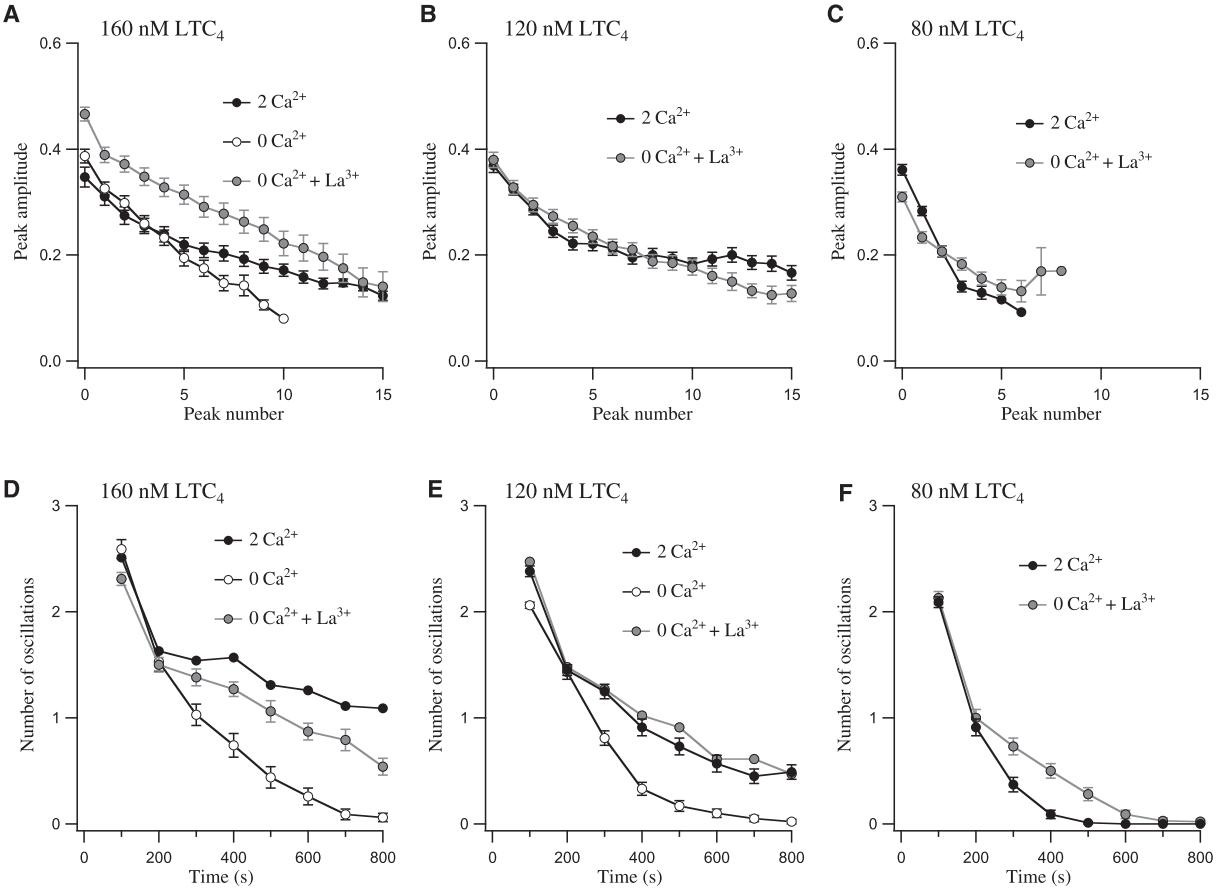

Figure 2. The Amplitude and Frequency of Ca^{2+} Oscillations to Three Different LTC_4 Concentrations Are Compared

(A–C) The amplitude of each Ca^{2+} oscillation against the oscillation number is plotted. The first Ca^{2+} oscillation is presented as peak 0, the second as peak 1, and so on.

(A) Response to 160 nM LTC_4 for the three different conditions shown.

(B and C) Response to 120 nM and 80 nM LTC_4, respectively.

(D–F) The number of Ca^{2+} oscillations over an 800 s time frame is plotted. Oscillations were binned in 100 s intervals.

(D) Response to 160 nM LTC_4.

(E and F) Response to 120 nM and 80 nM LTC_4, respectively.

Data are presented as mean ± SEM.

Stimulation of cysteinyl leukotriene type I receptors with LTC_4 activates CRAC channels [20] and a low concentration of Gd^{3+} (which is considered specific for CRAC channels) accelerated the decline of Ca^{2+} oscillations in 2 mM external Ca^{2+} (Figure S1). To confirm that Ca^{2+} entry through CRAC channels drives gene expression, we blocked the channels with the Synta compound and examined the effects on *c-fos* induction. As we have shown recently [25], the Synta compound is an effective inhibitor of CRAC channels. Stimulation with 160 nM LTC_4 in Ca^{2+}-free solution evoked a series of Ca^{2+} oscillations that ran down after ~7 min. Readmission of external Ca^{2+} resulted in a cytoplasmic Ca^{2+} rise resulting from Ca^{2+} influx through the open CRAC channels (Figure 4A). Pretreatment with 10 μM Synta compound substantially reduced this Ca^{2+} signal. Importantly, *c-fos* expression in response to LTC_4 (applied in the presence of 2 mM external Ca^{2+}) was prevented by the Synta compound (Figure 4B). Identical results were obtained with a lower concentration of LTC_4 (80 nM).

The finding that the amplitude and frequency of Ca^{2+} oscillations to LTC_4 were similar in 2 mM Ca^{2+} compared with 0 Ca^{2+} and La^{3+} suggests that Ca^{2+} entry and removal mechanisms are in balance. CRAC channels cluster in punctate-like structures upon store depletion [26], but it is not known whether the plasma membrane Ca^{2+} pumps or sarco-endoplasmic reticulum Ca^{2+}ATPase pumps in peripheral endoplasmic reticulum colocalize here too. Nevertheless, it is likely that Ca^{2+} removal pathways are positioned such that they prevent a significant global Ca^{2+} increase after Ca^{2+} entry, while impacting little on the local subplasmalemmal Ca^{2+} rise in the vicinity of open CRAC channels. Clustering of removal mechanisms might be one effective way to ensure that local Ca^{2+} entry is impeded from eliciting a more global Ca^{2+} rise [27].

We considered the possibility that La^{3+} might enter cells and interfere with *c-fos* expression, thus complicating interpretation of the experiments in Figure 3 where gene expression is measured in 0 Ca^{2+} plus La^{3+}. Two arguments can be raised against this. First, we have previously shown that La^{3+} does not enter cells to any detectable level over the time course of the experiments described here [18]. Second, we triggered Ca^{2+} influx independent of store-operated Ca^{2+} entry to see whether La^{3+} inhibited the subsequent activation of gene expression. We used the Ca^{2+} ionophore ionomycin, which increases Ca^{2+} entry directly through its ionophore properties

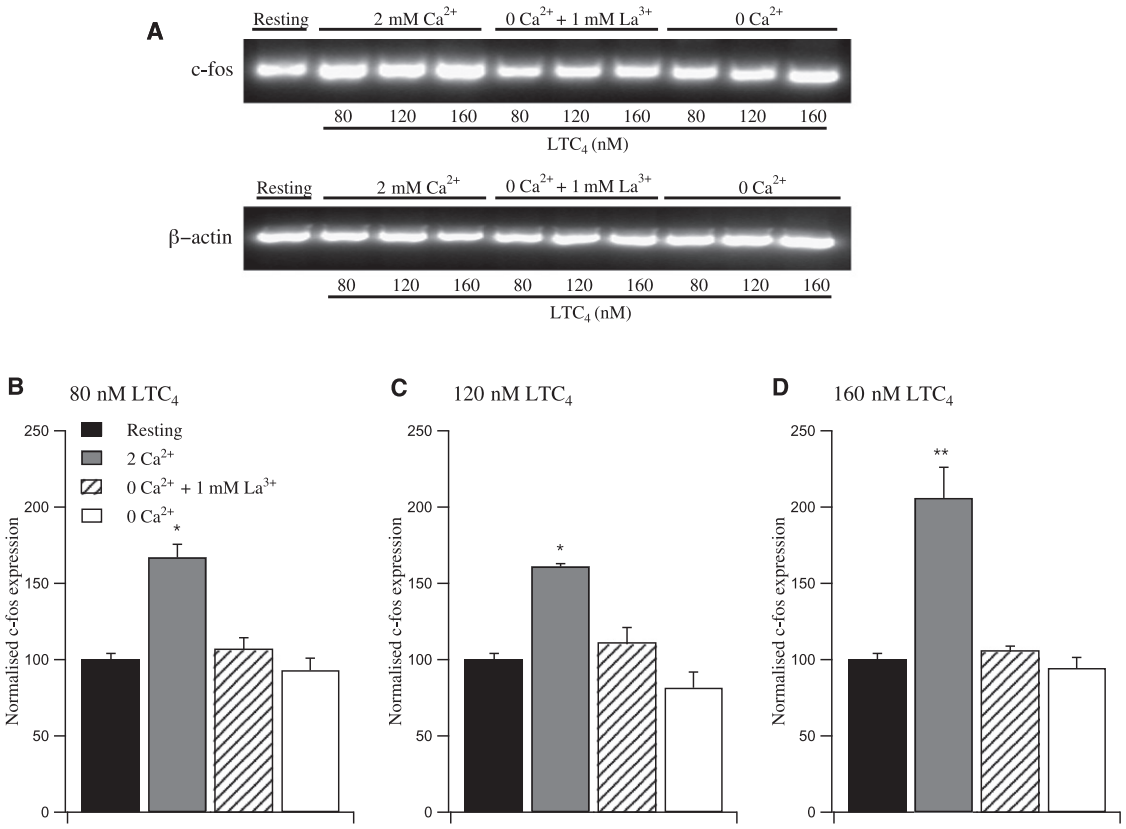

Figure 3. Gene Expression Occurs Only when Ca^{2+} Entry Accompanies the Oscillatory Signal

(A) *c-fos* expression is compared in cells exposed to different concentrations of LTC_4 in 2 mM Ca^{2+}, 0 Ca^{2+} and La^{3+}, or 0 Ca^{2+} alone.

(B–D) The extent of *c-fos* expression (normalized to control levels in nonstimulated cells) is compared for the different conditions after stimulation in 80 nM (B), 120 nM (C), and 160 nM LTC_4 (D). Over the concentration range tested, gene expression occurred only when accompanied by Ca^{2+} influx. Data are presented as mean ± SEM. *$p < 0.01$; **$p < 0.001$.

and indirectly through store depletion and subsequent activation of CRAC channels [28]. Because 1 mM La^{3+} completely abolishes store-operated Ca^{2+} entry (Figure 4C), we were able to isolate the Ca^{2+} influx because of direct ionophore transport by pretreating cells with this trivalent cation. The Ca^{2+} response to ionomycin was slightly reduced by 1 mM La^{3+} (Figure 4D) but *c-fos* expression was unaffected (Figure 4E). Hence La^{3+} does not interfere with Ca^{2+}-induced gene expression.

Are global Ca^{2+} oscillations necessary for driving gene expression or is local Ca^{2+} influx sufficient? To distinguish between these possibilities, we increased cytoplasmic Ca^{2+} buffering by loading cells with the slow Ca^{2+} chelator EGTA. EGTA reduces the bulk Ca^{2+} rise but is too slow to interfere with the amplitude or extent of local Ca^{2+} entry [11, 12]. As we have shown previously, preincubating cells with EGTA-AM substantially reduced the Ca^{2+} rise that occurred after store-operated Ca^{2+} entry [18]. EGTA also abolished the global Ca^{2+} oscillations evoked by LTC_4 (63 cells) but had no inhibitory effect on *c-fos* expression in response to the same concentration of agonist (Figure 4F). We considered the possibility that the *c-fos* expression response might have already saturated after stimulation with thapsigargin in 2 mM Ca^{2+}. If so, this could mask a potential difference in *c-fos* expression when compared with that induced after loading the cytoplasm with EGTA. However, a modest fall in the rate of Ca^{2+} entry produced a reduction in the extent of *c-fos* expression, arguing against saturation of the response. Readmission of 0.25 mM and 0.5 mM external Ca^{2+} to cells pretreated with thapsigargin in Ca^{2+}-free solution elicited a slower rate of Ca^{2+} influx (22.8% ± 6.7% and 48.9% ± 11.1% of that seen in 2 mM Ca^{2+} [18]). *c-fos* expression was likewise reduced to 38% ± 3.8% and 64% ± 5.1% ($p < 0.01$ in both cases) that seen in 2 mM Ca^{2+}.

The Ca^{2+} chelator BAPTA is fast enough to intercept incoming Ca^{2+} and thus reduce the amplitude and spatial extent of local Ca^{2+} entry [11]. Loading the cytoplasm with BAPTA suppressed gene expression induced by LTC_4 (Figure 4F). Because BAPTA is able to restrict Ca^{2+} influx to within a few nanometers of the channel pore, these results demonstrate that local Ca^{2+} influx drives gene expression and that global Ca^{2+} oscillations are not essential to this process.

Our findings provide new insight into how cells decode the universal oscillatory Ca^{2+} signal. Rather than responding to the amplitude or frequency of the Ca^{2+} oscillations, we have discovered that the spatial profile of the oscillatory Ca^{2+} signal is the key trigger for gene expression. Abolition of global Ca^{2+} oscillations by increasing cytoplasmic Ca^{2+} buffering had no inhibitory effect on gene expression. Instead, local Ca^{2+} influx through CRAC channels was sufficient to drive *c-fos* transcription. The digital nature of the Ca^{2+} oscillations is therefore not the fundamental signaling unit used to drive excitation-transcription coupling at low levels of stimulus intensity, at least in the cell type we have used.

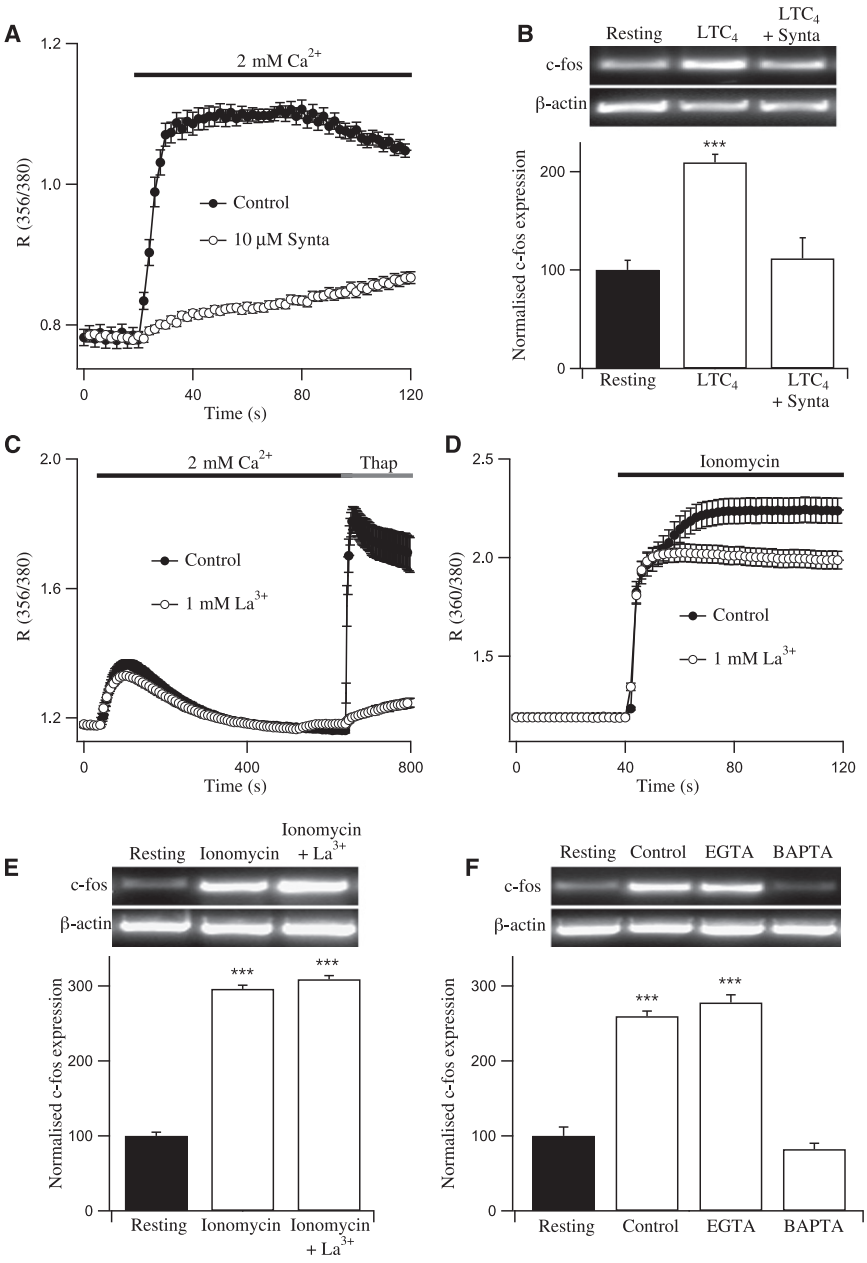

Figure 4. Gene Expression Can Occur in the Absence of Ca^{2+} Oscillations

(A) Cells were stimulated with LTC_4 (160 nM) in Ca^{2+}-free solution and once oscillations had stopped, 2 mM Ca^{2+} was applied. Store-operated Ca^{2+} entry occurred (filled circles, mean of >80 cells) and this was blocked by pretreatment with the Synta compound (10 μM, applied 5 min before Ca^{2+} was readmitted; >50 cells). Time 0 on the graph represents 60 s after cells had stopped oscillating.

(B) The Synta compound suppresses gene expression induced by LTC_4. Top: Gel shows *c-fos* induction by 160 nM LTC_4 and this is blocked by the Synta compound. Lower panel summarizes aggregate data from three such experiments.

(C) 1 mM La^{3+} abolishes store-operated Ca^{2+} influx. Thapsigargin (2 μM) was applied in 0 Ca^{2+} external solution to deplete the stores and then 2 mM external Ca^{2+} was readmitted as indicated.

(D) The Ca^{2+} signal evoked by 10 μM ionomycin was only partially reduced by La^{3+}.

(E) *c-fos* expression was induced by ionomycin and this was unaffected by La^{3+}.

(F) Loading cells with EGTA, which prevented the oscillatory response, had no effect on gene expression to LTC_4 whereas loading with BAPTA suppressed *c-fos* induction. Control refers to LTC_4 in 2 mM Ca^{2+}.

Data are presented as mean ± SEM. ***p < 0.0005.

Although the importance of CRAC channels to physiological responses has been appreciated, it was thought that the role of the channels was simply to provide a "top-up" of Ca^{2+} stores, remedying the small loss of Ca^{2+} from the cell after each Ca^{2+} oscillation. Our data clearly reveal that the global Ca^{2+} rise associated with Ca^{2+} oscillations does not provide the Ca^{2+} that activates gene expression; rather, it is the spatially localized Ca^{2+} signal that arises from open CRAC channels that triggers the nuclear response. Hence CRAC channels do not simply support the signaling function of Ca^{2+} oscillations. On the contrary, it appears that the function of Ca^{2+} oscillations is to activate CRAC channels, which provides the important and physiological Ca^{2+} signal, at least for *c-fos* gene regulation.

The frequency of oscillatory Ca^{2+} signals can recruit different transcription factors to the nucleus [5], alter mitochondrial ATP production [6], and drive exocytosis [10]. Our results now show that important information is contained in the spatial signature of the Ca^{2+} oscillation. In neurons, spatially restricted Ca^{2+} signals at the cell periphery can influence nuclear events: Ca^{2+} microdomains arising from L-type Ca^{2+} channels [15, 29] or NMDA receptors [30] activate gene expression independent of the bulk Ca^{2+} rise. Although these pioneering studies revealed the importance of local Ca^{2+} signals in driving excitation-transcription coupling, the stimuli used were 60–90 mM K^+ pulses for tens of seconds or bursts of action potentials for 5 min. In our experiments, we have used a physiologically relevant trigger, namely a low dose of an agonist. Spatially restricted local Ca^{2+} entry can therefore trigger transcription after a stimulus of physiological intensity. Moreover, it is this spatial signature within a global Ca^{2+} oscillation, rather than the oscillation itself, that triggers *c-fos* gene expression. Such spatial decoding greatly increases the versatility of Ca^{2+} as a second messenger, enhances the efficacy of Ca^{2+} in promoting gene expression, and provides a novel means for generating specific Ca^{2+}-dependent cellular responses.

Experimental Procedures

Cell Culture

The mast cell line RBL-1 was bought from ATCC and cultured as previously described [25].

Single-Cell Ca^{2+} Imaging

RBL-1 cells were loaded with fura 5F as described [18]. Cells were excited at 356 and 380 nm (20 ms exposures) at 0.5 Hz and the ratio (R) calculated.

Standard external solution contained (in mM): NaCl 145, KCl 4.5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, D-glucose 10 (pH 7.2 with NaOH). In 0 Ca^{2+} solution, external Ca^{2+} was removed and 0.1 mM EGTA (pH 7.2 with NaOH) was added. 0 Ca^{2+} + La^{3+} was identical to 0 Ca^{2+} but 1 mM La^{3+} was added (and the pH returned to 7.2 with NaOH because EGTA can bind La^{3+}). LTC_4 was purchased from Axxora.

EGTA and BAPTA Loading

Cytoplasmic loading with EGTA or BAPTA was carried out by preincubating cells with EGTA-AM or BAPTA-AM (25 μM) for 40 min at ~24°C in the dark together with fura 5F (2 μM). Cells were then washed three times and allowed to recover for a further 15 min in the dark.

c-fos Expression

RT-PCR was carried out as described previously [24].

Supplemental Data

Supplemental Data include one figure and can be found with this article online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)00917-8.

Acknowledgments

This work was supported by an MRC programme grant to A.B.P. J.D.C. held a Christopher Welch Scholarship. S.-W.N. holds a BBSRC-GlaxoSmithKline (GSK) studentship. We thank GSK for the synthesis and provision of the Synta compound.

Received: January 20, 2009
Revised: March 19, 2009
Accepted: March 19, 2009
Published online: April 16, 2009

References

1. Berridge, M.J., Bootman, M.D., and Roderick, H.L. (2003). Calcium signalling: Dynamics, homeostasis and remodelling. Nat. Rev. Mol. Cell Biol. *4*, 517–529.
2. Clapham, D.E. (2008). Calcium signaling. Cell *131*, 1047–1058.
3. Petersen, O.H. (2005). Calcium signalling and calcium-activated ion channels in exocrine acinar cells. Cell Calcium *38*, 171–200.
4. Dolmetsch, R.E., Lewis, R.S., Goodnow, C.C., and Healy, J.I. (1997). Differential activation of transcription factors induced by calcium response amplitude and duration. Nature *386*, 855–858.
5. Dolmetsch, R.E., Xu, K., and Lewis, R.S. (1998). Calcium oscillations increase the efficiency and specificity of gene expression. Nature *392*, 933–936.
6. Hajnoczky, G., Robb-Gaspers, L.D., Seitz, M.B., and Thomas, A.P. (1995). Decoding of cytosolic calcium oscillations in the mitochondria. Cell *82*, 415–424.
7. Prince, W.T., and Berridge, M.J. (1973). The role of calcium in the action of 5-hydroxytryptamine and cyclic AMP on salivary glands. J. Exp. Biol. *58*, 367–384.
8. Berridge, M.J. (1990). Calcium oscillations. J. Biol. Chem. *265*, 9583–9586.
9. Thomas, A.P., Bird, G.S., Hajnoczky, G., Robb-Gaspers, L.D., and Putney, J.W.J. (1996). Spatial and temporal aspects of cellular calcium signalling. FASEB J. *10*, 1505–1517.
10. Tse, A., Tse, F.W., Almers, W., and Hille, B. (1993). Rhythmic exocytosis stimulated by GnRH-induced caclium oscillations in rat gonadotropes. Science *260*, 82–84.
11. Neher, E. (1998). Vesicle pools and Ca^{2+} microdomains: New tools for understanding their roles in neurotransmitter release. Neuron *20*, 389–399.
12. Parekh, A.B. (2008). Ca^{2+} microdomains near plasma membrane Ca^{2+} channels: Impact on cell function. J. Physiol. *586*, 3043–3054.
13. Berkefeld, H., Sailer, C.A., Bildl, W., Rhode, V., Thumfart, J.O., Eble, S., Klugbauer, N., Reisinger, E., Bischofberger, J., Oliver, D., et al. (2006). BKCa-CaV channel complexes mediate rapid and localized calcium-activated potassium signalling. Science *314*, 615–620.
14. Deisseroth, K., Bito, H., and Tsien, R.W. (1996). Signaling from synapse to nucleus: postsynaptic CREB phosphorylation during multiple forms of hippocampal synaptic plasticity. Neuron *16*, 89–101.
15. Dolmetsch, R.E., Pajvani, V., Fife, K., Spotts, J.M., and Greenberg, M.E. (2001). Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. Science *294*, 333–339.
16. Bautista, D.M., and Lewis, R.S. (2004). Modulation of plasma membrane calcium-ATPase activity by local calcium microdomains near CRAC channels in human T cells. J. Physiol. *556*, 805–817.
17. Willoughby, D., and Cooper, D.M.F. (2007). Organization and Ca^{2+} regulation of adenylyl cyclases in cAMP microdomains. Physiol. Rev. *87*, 965–1010.
18. Chang, W.C., Di Capite, J., Singaravelu, K., Nelson, C., Halse, V., and Parekh, A.B. (2008). Local calcium influx through calcium release-activated calcium (CRAC) channels stimulates production of an intracellular messenger and an intercellular pro-inflammatory signal. J. Biol. Chem. *283*, 4622–4631.
19. Rizzuto, R., and Pozzan, T. (2006). Microdomains of intracellular calcium: Molecular determinants and functional consequences. Physiol. Rev. *86*, 369–408.
20. DiCapite, J.L., Shirley, A., Nelson, C., Bates, G., and Parekh, A.B. (2009). Intercellular calcium wave propagation involving positive feedback between CRAC channels and cysteinyl leukotrienes. FASEB J. *23*, 894–905.
21. Moreau, B., Straube, S., Fisher, R.J., Putney, J.W., and Parekh, A.B. (2005). Ca^{2+} -calmodulin-dependent facilitation and Ca^{2+} inactivation of Ca^{2+} release-activated Ca^{2+} channels. J. Biol. Chem. *280*, 8776–8783.
22. Bird, G.S., and Putney, J.W.J. (2005). Capacitative calcium entry supports calcium oscillations in human embryonic kidney cells. J. Physiol. *562*, 697–706.
23. Lee, Y.-N., Tuckerman, J., Nechushtan, H., Schutz, G., Razin, E., and Angel, P. (2004). *c-fos* as a regulator of degranulation and cytokine production in FCeRI-activated mast cells. J. Immunol. *173*, 2571–2577.
24. Chang, W.-C., Nelson, C., and Parekh, A.B. (2006). Ca^{2+} influx through CRAC channels activates cytosolic phospholipase A2, leukotriene C4 secretion and expression of *c-fos* through ERK-dependent and independent pathways in mast cells. FASEB J. *20*, 2381–2383.
25. Ng, S.-W., DiCapite, J.L., Singaravelu, K., and Parekh, A.B. (2008). Sustained activation of the tyrosine kinase Syk by antigen in mast cells requires local Ca^{2+} influx through Ca^{2+} release-activated Ca^{2+} channels. J. Biol. Chem. *283*, 31348–31355.
26. Lewis, R.S. (2007). The molecular choreography of a store-operated calcium channel. Nature *446*, 284–287.
27. Petersen, O.H., and Tepikin, A. (2008). Polarized calcium signalling in exocrine gland cells. Annu. Rev. Physiol. *70*, 273–299.
28. Vig, M., DeHaven, W.I., Bird, G.S., Billingsley, J.M., Wang, H.J., Rao, P.E., Hutchings, A.B., Jouvin, M.H., Putney, J.W., and Kinet, J.P. (2008). Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. Nat. Immunol. *9*, 89–96.
29. Deisseroth, K., Heist, E.K., and Tsien, R.W. (1998). Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. Nature *392*, 198–202.
30. Hardingham, G.E., Arnold, F.J.L., and Bading, H. (2001). A calcium microdomain near NMDA receptors: On switch for ERK-dependent synapse-to-nucleus communication. Nat. Neurosci. *4*, 565–566.